SUCCINOXIDASE OF POTATO TUBER.

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(Two Text-figures.)

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Synopsis.

The participation of a tricarboxylic acid cycle, similar to that of animal tissues, in the aerobic metabolism of plants would imply the participation of succinic dehydrogenase, an enzyme which, in animal tissues, is coupled with the cytochrome system, the whole forming the complex known as succinoxidase. Investigations carried out with potato (*Solanum tuberosum* L.) tuber have shown that this tissue contains an active succinoxidase system, that the system is associated with particulate components of the cytoplasm and that succinic dehydrogenase is coupled to the cytochrome system in the plant as in the succinoxidase of animal tissues. Also associated with these particles, which resemble and may be identical with mitochondria, are the enzymes, fumarase and malic dehydrogenase.

Introduction.

The participation of a tricarboxylic acid cycle, similar to that of animals tissues, in the aerobic metabolism of plants would imply the participation of succinic dehydrogenase, an enzyme, which, in animal tissues, is coupled with the cytochrome system, the whole forming the complex known as succinoxidase. The *in vitro* demonstration of succinic dehydrogenase activity in plant material has, however, been accomplished in relatively few instances. Okunuki (1939) found succinoxidase activity in pollen of *Lilium auratum*. Slight succinic dehydrogenase activity has been detected in extracts of seedlings of some Leguminosae (Damodaran and Ramaswamy, 1940). Damodaran and Venkatesen (1941) demonstrated the succinoxidase system in preparations of young seedlings and pods of certain Leguminosae and examined in detail an active preparation of the enzyme from seedlings of *Phaseolus mungo*. Goddard (1944) has also reported a low succinic dehydrogenase activity associated with cytochrome oxidase preparations from wheat germ.

From an examination of the effect of malonate on the accumulation of succinate, Bonner (1948) concluded that succinic dehydrogenase must function in the metabolism of pyruvate by segments of *Avena* coleoptile. Laties (1949) has also demonstrated the accumulation of succinate in the presence of malonate in spinach leaves and in excised barley roots.

The purpose of the present investigation was the study of the succinoxidase system of the potato tuber both in relation to succinic dehydrogenase and in relation to the cytochrome system. During the course of the investigation work bearing on this problem has appeared from other laboratories. Cytochrome oxidase activity in preparations from potato tuber has been demonstrated by Levy and Schade (1948) and studied in detail by Goddard and Holden (1950).

In general the results of the present work show, as do those of Levy and Schade and of Goddard and Holden, that potato tubers contain an active succinoxidase system, that the system is associated with particulate components of the cytoplasm, and that succinic dehydrogenase is coupled to the cytochrome system in the plant as in the succinoxidase of animal tissues.

Plant Material.

Mature tubers from *Solanum tuberosum* L. (variety: Factor, grown at Blayney, N.S.W.) were used throughout. Fifty pounds of freshly harvested tubers were stored at approximately 15°C. and aliquots removed as needed. No systematic differences in

succinoxidase activity were noticed during the storage period of five months, nor were differences found between potatoes from the two successive harvests used during the investigation.

Reagents.

Cytochrome c was prepared from horse cardiac muscle according to the method of Keilin and Hartree (1937), but was dialysed against distilled water rather than against 1% sodium chloride (Potter, 1941). The concentration was determined by measuring the absorption at 550mμ after oxidation with ferricyanide and reduction with hydrosulphite.

Calcium cyanide was made according to Robbie and Leinfelder (1945) and estimated gravimetrically by precipitation as silver cyanide.

Phosphate buffers (a mixture of Na_2HPO_4 and KH_2PO_4) were used throughout. Hydrogen ion determinations were made by means of a Leeds and Northrop glass electrode pH meter.

All solutions were made from A.R. chemicals.

Substrates and inhibitors were neutralized to litmus with sodium hydroxide where necessary.

Methods.

Succinoxidase activity was followed by measurement of oxygen uptake according to the standard Warburg manometric technique. Potassium hydroxide was employed in the centre well for absorption of carbon dioxide. In an examination of the effect of cyanide on the aerobic system, calcium cyanide–calcium hydroxide mixtures (Robbie, 1946) were employed. Carbon dioxide output was measured by the direct method of Warburg. Determination of dehydrogenase activity was done by the Thunberg technique carried out in an atmosphere of nitrogen. Estimates of activity were based on the time needed for complete decoloration of the redox dye used. All experiments were conducted at 37°C. Succinic and fumaric acids were estimated quantitatively according to Krebs *et al.* (1940). Chromatographic identification of organic acids followed the procedure of Lugg and Overell (1948).

Preparation of Enzyme.

The tubers were first scrubbed in running tap-water, immersed for one minute in a saturated, filtered solution of bleaching powder (CaClOCl), and thoroughly rinsed in tap-water. The potato (300 gm.) was then diced into 100 ml. of 0·02M phosphate buffer (pH 10·2). The diced material was next ground for one minute in a mechanical macerator (overhead-drive Waring blendor). The pH of the mixture was maintained at approximately 7·2 during this operation by the addition of 1N sodium hydroxide, bromthymol blue being employed as an external indicator. The brei was now filtered through muslin and allowed to stand in the refrigerator for ten minutes, after which the supernatant was decanted from the sedimented starch and centrifuged at approximately 1700g for ten minutes. The supernatant from the centrifugation was removed by decantation and the residue, which contained the enzyme, suspended in 20ml. of 0·01M Na_2HPO_4 for experiments on succinoxidase and in 20ml. of 0·01M KH_2PO_4 for experiments on succinic dehydrogenase activity. Two millilitres of this preparation, containing 0·8–1·0mg. total nitrogen, was used in each experimental vessel.

All vessels and solutions employed were chilled before use and the entire preparation of the enzyme was carried out at a temperature of 5°C. or below.

Experimental.

Demonstration of Succinic Dehydrogenase Activity.

Potato tubers contain an active succinic dehydrogenase as judged by ability to catalyse the reduction of 2,6-dichlorphenolindophenol at the expense of succinate under anaerobic conditions in the Thunberg technique. The enzyme is an insoluble one, bound to particles which are sedimented in 10 min. in a field of 1700g, as noted above. Microscopic observations show that the preparation as described above consists of particles roughly 1μ in diameter.

The standard method adopted for preparation of the particle-bound enzyme involved sedimentation by centrifugation from the extract at pH 7·2. The pH of the extract before and during centrifugation is critical, since it exerts an effect on the amount of

TABLE 1.

Effect of pH during Preparation on the Endogenous Substrate Level of Succinic Dehydrogenase from Potato Tuber.

Main tube: 2·0 ml. enzyme, 0·5 ml. 0·2 M phosphate buffer (pH 7·3), 0·1 ml. 0·2 M succinate or 0·1 ml. water.
Sidearm: 0·3 ml. 0·05% 2,6-dichlorphenolindophenol.

	pH of Extract.	Decoloration Time (Min.).	
		No Added Substrate.	Added Succinate.
Expt. 1 ..	7·96	100	4·5
	7·53	100	4·5
	6·48	100	5
Expt. 2 ..	6·48	55	3·5
	6·00	20	4
Expt. 3 ..	6·02	33	4
	5·76	21	4
	5·42	9	3
Expt. 4 ..	5·35	3	1
	5·02	1	1
	4·46	1	1
	3·98	1	1
	3·44	1	1

endogenous substrate contained in the enzyme preparation. The data of Table 1 show that when the extract is maintained at acidities greater than pH 5, the enzyme preparation obtained contains material which causes the rapid reduction of the redox dye in the Thunberg technique even without the addition of further substrate. This effect is minimized when the extract is maintained at pH values above 7.

Even preparations made by grinding potato tubers in water contain some succinic dehydrogenase activity, as is shown in Table 2. The preparation for this experiment was made by treating diced potato with one-third of its weight of distilled water for

TABLE 2.

Succinic Dehydrogenase Activity of Enzyme Prepared by Maceration of Tissue in Distilled Water.

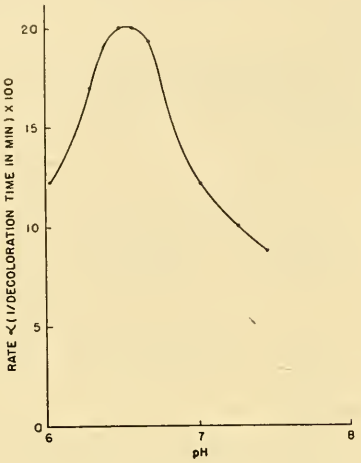
Main tube: 2·0 ml. enzyme, 0·5 ml. 0·2 M phosphate buffer (pH 7·3), 0·1 ml. 0·2 M succinate or 0·1 ml. water.
Sidearm: 0·3 ml. 0·05% 2,6-dichlorphenolindophenol.

	Decoloration Time (Min.).
Control	35
+Succinate	8

two minutes in a Waring blendor. The enzyme was then separated by centrifugation as described above.

pH.—The succinic dehydrogenase of potato was found to have a pH optimum of 6·5 in phosphate buffer, as shown in Text-figure 1. Succinic dehydrogenase from muscle (Ohlsson, 1921) and from bacteria (Cook and Alcock, 1931) has been shown to

demonstrate maximal activity, as measured by the rate of decoloration of methylene blue at pH 9.



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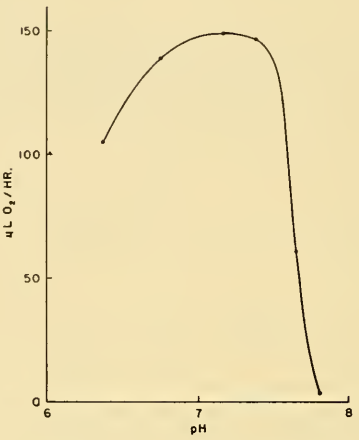

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Text-figure 1.—Effect of pH on succinic dehydrogenase activity. Main tube: 2·0ml. enzyme, 0·5ml. 0·2M phosphate buffer, 0·1ml. 0·2M succinate. Sidearm: 0·3ml. 0·05% 2,6-dichlorphenolindophenol.

Text-figure 2.—Effect of pH on succinoxidase activity. Each flask contained 2·0ml. enzyme and 0·5ml. 0·2M phosphate buffer. The sidearm contained 0·2ml. cytochrome c (final concentration 2×10^{-5}M), 0·1ml. 0·2M succinate or water to 0·3ml.

Redox Dyes.—The activity of potato succinic dehydrogenase as measured in the Thunberg technique is markedly affected by the nature of the redox dye employed (Table 3). Methylene blue and thionine were relatively ineffective as hydrogen acceptors, while 2,6-dichlorphenolindophenol proved quite effective.

TABLE 3.

Effect of Nature of Redox Dye on Succinic Dehydrogenase Activity.

Main tube: 2·0 ml. enzyme, 0·5 ml. 0·2 M phosphate buffer (pH 6·5), 0·1 ml. water, 0·1 ml. 0·2 M succinate or 0·1 ml. water.
Sidearm: 0·3 ml. dye.

Redox Dye.	Conc. (M.).	Decoloration Time (Min.).	
		No Added Substrate.	Added Succinate.
2,6-dichlorphenolindophenol ..	5×10^{-3}	No apparent change in 15 hours.	
	10^{-3}	120	33
	10^{-4}	75	2·2
	10^{-5}	0·2	0·08
Thionine (Lauth's violet) ..	10^{-3}	No apparent change in 240 min.	
	10^{-4}	240	50
	10^{-5}	10	9
Methylene blue	10^{-3}	No apparent change in 240 min.	
	10^{-4}	,, ,,	,,
	10^{-5}	,, ,,	,,

On the basis of the data of Table 3, 2,6-dichlorphenolindophenol at a final concentration of 10^{-4}M was chosen for all further Thunberg experiments.

Succinate Concentration.

Data on the relation of potato succinic dehydrogenase activity to substrate concentration are presented in Table 4. The Michaelis-Menten constant, Km, derived from

these data, is $1{\cdot}9 \times 10^{-3}$M. The Km for muscle succinic dehydrogenase has been found to be 1×10^{-3}M (Lardy, 1949).

TABLE 4.

Effect of Concentration of Succinate on Succinic Dehydrogenase Activity.

Main tube: 2·0 ml. enzyme, 0·5 ml. 0·2 M phosphate (pH 6·5), 0·1 ml. water, 0·1 ml. succinate or 0·1 ml. water.
Sidearm: 0·3 ml. 10^{-3} M 2,6-dichlorphenolindophenol.

Succinate Concentration (M.)	Decoloration Time (Min.).
	75
$6{\cdot}67 \times 10^{-2}$	3·3
$3{\cdot}33 \times 10^{-2}$	2·5
$1{\cdot}67 \times 10^{-2}$	3
$6{\cdot}67 \times 10^{-3}$	3·5
$3{\cdot}33 \times 10^{-3}$	4·5
$1{\cdot}67 \times 10^{-3}$	6·2
$6{\cdot}67 \times 10^{-4}$	10·5
$3{\cdot}33 \times 10^{-4}$	36

Inhibitors.—Succinic dehydrogenase of animal tissues is known to be competitively inhibited by malonate (Krebs and Eggleston, 1940) and to be sensitive to SH-reagents (Potter and DuBois, 1943). Succinic dehydrogenase of potato tuber was likewise inhibited by malonate as well as by phenylmercuric acetate, a potent SH-reagent. It was not, however, inhibited by iodoacetate, a relatively weak SH-reagent (Table 5).

TABLE 5.

Effect of Varied Inhibitors on Potato Succinic Dehydrogenase.

Main tube: 2·0 ml. enzyme, 0·5 ml. 0·2 M phosphate buffer (pH 6·5), 0·1 ml. 0·2 M succinate, 0·3 ml. inhibitor or 0·3 ml. water.
Sidearm: 0·3 ml. 10^{-3} M 2,6-dichlorphenolindophenol.

Inhibitor.	Conc. (M.).	Decoloration Time (Min.).
Phenylmercuric acetate	—	1·2
	10^{-3}	75
	2×10^{-4}	75
	10^{-4}	24
	2×10^{-5}	2·3
	10^{-6}	1·8
	10^{-6}	1·8
Malonate	—	1·5
	10^{-2}	5·5
	10^{-3}	2·1
	10^{-4}	1·5
	10^{-5}	1·5
	10^{-6}	1·5
Indoacetate	—	1
	10^{-2}	1
	10^{-3}	1
	10^{-4}	1
	10^{-5}	1
	10^{-6}	1
Cyanide	—	2
	10^{-2}	0·8*
	10^{-3}	1*
	10^{-4}	2
	10^{-5}	2
	10^{-6}	2

* The slight increase in the rate of decoloration of the redox dye as noted here, was shown by control experiments to be due to an effect of the inhibitor in the absence of added substrate.

Demonstration of Succinoxidase Activity.—The potato enzyme preparation described above is not only able to oxidize succinate anaerobically, but is also able to oxidize succinate aerobically with the uptake of oxygen. This oxidation is markedly increased by the addition of cytochrome c to the reaction mixture, as is shown in Table 6. Succinic dehydrogenase of potato tuber appears therefore to be, like the corresponding enzyme from animal tissue, linked with the cytochrome oxidase system and may thus be referred to as a succinoxidase.

TABLE 6.

Presence of Succinoxidase in Particulate Potato Succinic Dehydrogenase.

Each flask contained 2·0 ml. enzyme and 0·5 ml. 0·2 M phosphate buffer (pH 7·0).

The sidearm contained 0·2 ml. cytochrome c (final concentration 2×10^{-5} M), 0·1 ml. 0·2 M succinate or water to 0·3 ml.

	µl. O_2 Uptake per Hour.
Control (no substrate, no cytochrome)	0
+cytochrome c	4
+succinate	11
+cytochrome c+succinate ..	80

pH.—The succinoxidase of potato tuber shows a pH optimum in phosphate buffer of 7·2 (Text-fig. 2). The system as a whole thus has a pH optimum different from that of succinic dehydrogenase, which has been shown above (Text-fig. 1) to be *ca.* 6·5. The pH optimum for muscle succinoxidase has been found to lie in the range pH 7·3–7·8 (Wieland and Frage, 1929).

Inhibitors.—The succinoxidase system is sensitive to phenylmercuric acetate, malonate, cyanide and iodoacetate (Table 7). Comparison of the results of Tables 5 and 7 shows that phenylmercuric acetate is an effective inhibitor of both succinic dehydrogenase and of the complete succinoxidase system. The complete system is even inhibited by concentrations of phenyl-mercuric acetate (10^{-5}M), which have little effect on dehydrogenase activity. Malonate at high concentrations (10^{-2}M and 10^{-3}M) is an effective inhibitor of both dehydrogenase and oxidase activity. Again, however, at a concentration of 10^{-4}M, which exerts no effect on the dehydrogenase, some inhibition of the succinoxidase system is still observed. Cyanide has no inhibitory effect on succinic dehydrogenase activity, but is an effective inhibitor of succinoxidase presumably exerting its effect on cytochrome oxidase. Iodoacetate slightly inhibits the succinoxidase system but has no effect on dehydrogenase activity.

Since phenylmercuric acetate is known to inhibit enzyme systems by its effect on sulphydryl groups, the possibility of reactivating these groups by glutathione, as suggested by Barron and Singer (1945) was examined. The results of these attempts were, however, entirely negative (Table 8).

Fate of Metabolized Succinate.—The disappearance of succinate from a reaction mixture containing potato succinoxidase may be shown to correspond quantitatively to the reaction:

$$\text{Succinic acid} \xrightarrow{-2H+1/2O_2} \text{Fumaric acid} \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} \text{Malic acid}$$

(1) (2)

The increased oxygen uptake displayed by the system in the presence of succinate and cytochrome c takes place without concurrent increase in the small endogenous carbon dioxide evolution (Table 9). It must be concluded, therefore, that although the succinate is oxidized by the system it is not dismembered through further reactions of the Kreb's cycle.

TABLE 7.

Effect of Inhibitors on Succinoxidase Activity.

Each flask contained 2·0 ml. enzyme, 0·5 ml. 0·2 M phosphate buffer (pH 7·2), and 0·3 ml. inhibitor or 0·3 ml. water. The sidearm contained 0·1 ml. cytochrome c (final concentration 10^{-5} M) and 0·1 ml. 0·2 M succinate.

Inhibitor.	Concentration (M.).	μl. O_2 Uptake per Hour.	% Inhibition.
Phenylmercuric acetate ..	—	32	
	10^{-3}	0	100
	10^{-4}	0	100
	10^{-5}	17	47
	10^{-6}	28	13
Malonate	—	81	
	10^{-2}	16	80
	10^{-3}	44	46
	10^{-4}	49	40
	10^{-5}	62	24
	10^{-6}	66	19
Cyanide	—	100	
	10^{-3}	12	88
	10^{-4}	30	70
	10^{-5}	54	46
Iodoacetate	—	81	
	2×10^{-2}	60	26
	10^{-2}	69	15
	10^{-3}	75	7
	10^{-4}	76	6

TABLE 8.

Effect of Glutathione on Inhibition of Succinoxidase by Phenylmercuric Acetate.

Each flask contained 2·0 ml. enzyme and 0·5 ml. 0·2 M phosphate buffer (pH 7·2). The sidearm contained 0·1 ml. cytochrome c (final concentration 10^{-5} M), 0·1 ml. 0·2 M succinate, 0·1 ml. phenylmercuric acetate (final concentration 10^{-5} M), 0·1 ml. glutathione or water to 0·5 ml.

	μl. O_2 per Hour.
Control	0
+succinate+cytochrome c	40
+succinate+cytochrome c+phenylmercuric acetate	24
+succinate+cytochrome c+phenylmercuric acetate+glutathione (10^{-2} M)	28*
+succinate+cytochrome c+phenylmercuric acetate+glutathione (10^{-3} M)	15*
+succinate+cytochrome c+phenylmercuric acetate+glutathione (10^{-4} M)	16*

* These figures have been corrected for autoxidation of glutathione under the conditions of the experiment.

TABLE 9.

Oxidation of Succinate: Examination of Oxygen Absorption and Carbon Dioxide Evolution.

Each flask contained 2·0 ml. enzyme and 0·5 ml. 0·2 M phosphate buffer (pH 7·2). The sidearm contained 0·2 ml. cytochrome c (final concentration 2×10^{-5} M), 0·1 ml. 0·2 M succinate or water to 0·3 ml.

	−μl. O_2 per Hour.	+μl. CO_2 per Hour.
Control	0	5
+succinate	0	7
+succinate+cytochrome c	56	7

Identification of Reaction Products.—Identification of the actual oxidation products (Table 10) was carried out as follows. The aerobic oxidation of succinate was followed manometrically in the usual fashion. At the end of the experimental period (one hour) the manometer flasks were removed from the bath and to each cup was added 1ml. 5% metaphosphoric acid and the contents mixed. The contents of each flask were filtered through No. 1 Whatman filter paper, the flask washed repeatedly with small quantities (2–3ml.) water and the washings collected. The filtrate and washings were pooled and divided into two portions. On one portion succinic acid was estimated with the aid of heart muscle succinoxidase. The other portion was reduced in such a way as to convert any fumaric acid to succinic and the total succinic and fumaric acids then determined as succinic acid by the muscle preparation (Krebs *et al.*, 1940).

TABLE 10.

Quantitative Examination of the Fate of Added Succinate.

Eash flask contained 2·0 ml. enzyme (total nitrogen 0·8 mg.) and 0·5 ml. 0·2 M phosphate buffer (pH 7·2). The sidearm contained 0·1 ml. cytochrome c (final concentration 10^{-5} M), 0·2 ml. 0·2 M succinate or water to 0·3 ml.

	Succinate Added (Mg.).	μl. O_2 Absorbed in One Hour.	Succinate Disappearance Corresponding to Observed Oxygen Consumption (Mg.).	Succinate Disappearance (Analytical) (Mg.).
Control	0	5	0	0
+cytochrome c	0	7	0	0
+succinate	4·4	23*	0·19	0·2
+succinate+cytochrome c ..	4·4	132	1·39	1·4

* The enzyme preparation employed in the final section of this paper was prepared by centrifugation at −5° C.; the lower temperature maintained during the preparation resulted in an enzyme with some succinoxidase activity without added cytochrome c.

The validity of this procedure was established by recovery experiments carried out under the conditions of the experiment with succinic and fumaric acids added in amounts such as might be expected to be present. The method is accurate to ±0·1mg. succinic acid under the conditions of the experiment, and 80–90% recovery was obtained with added fumarate.

TABLE 11.

Identification of Organic Acids formed during the Aerobic Oxidation of Succinate.

	Rf Values of Plant Acids.		
	Acid Tested Separately.	Acid in Mixture.	Acid Detected in Reaction Mixture.
Succinic	0·701	0·694	0·687
Fumaric	0·818	0·811	0·817
Malic	0·448	0·444	0·449

Solvent: n-butanol/formic acid/water. Chromatographic separation carried out at 4° C.

If only reaction (1) were involved, fumaric acid should be present in amounts that would be readily estimated by the method used. Fumaric acid did not, however, appear as expected. On the contrary, only small increases in succinate concentration after

reduction were obtained. It was, therefore, suspected that fumarase might be present in the system and might then convert the fumarate formed to an equilibrium mixture of fumarate and malate (Krebs *et al.*, 1940). That this is indeed the case and that both fumarate and malate are products of the oxidation of succinic acid by the potato enzyme preparation was demonstrated with the aid of paper chromatography (Table 11).

The aerobic oxidation of succinate was followed by the Warburg technique. At the end of the experimental period (one hour) the flask contents were acidified (pH 2) with sulphuric acid and extracted with ether in a continuous extractor for thirty-six hours. The ethereal extract was concentrated, 2·0ml. water added, the ether removed by heating on a water bath, and the residue concentrated to approximately 1ml. Ten μl. of this extract was then applied to one end of a strip of filter paper and the chromatogram developed with butanol saturated with formic acid. The control (no added substrate) from the Warburg experiment was also extracted and examined in the same manner but no organic materials were detected. Comparable Rf values for succinic, fumaric and malic acids were determined by parallel experiments with the individual acids as well as with a mixture of the three acids. Twenty μg. of each acid was used in each case.

Discussion.

It has become increasingly evident that the particulate fraction of the cytoplasm is intimately concerned with the respiratory activity of animal tissues, since on these particles, the mitochondria, are located *inter alia*, all the enzymes necessary for the metabolism of pyruvate to carbon dioxide and water *via* the Krebs cycle (Green *et al.*, 1948). It is of interest, therefore, that in plants also it can be demonstrated that some at least of the enzymes participating in the tricarboxylic acid cycle are located on particles present in the cytoplasm—particles which would appear to correspond to the mitochondria of animal tissues. It has been possible to prepare particles similar to those described in this paper but which possess malic dehydrogenase activity in addition to the enzymes succinic dehydrogenase, fumerase and cytochrome oxidase. It will be of interest, therefore, to determine if further enzymes of the cycle are located on these particles and whether or not particles may be prepared from plant tissues which are capable of carrying out the complete oxidation of pyruvate by way of the Krebs cycle.

Summary.

An enzyme complex has been prepared from potato tuber which is capable of converting succinic acid to a mixture of fumaric and malic acids. In this complex the conversion of succinic acid to fumaric aid is coupled to the uptake of molecular oxygen *via* the cytochrome system. The enzyme complex is associated with particles which resemble and may be identical with the mitochondria.

Acknowledgement.

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References.

Barron, E. S. G., and Singer, T. P., 1945.—Studies on biological oxidations. 9. Sulfhydryl enzymes in carbohydrate metabolism. *J. Biol. Chem.*, 157 : 221-240.

Bonner, J., 1948.—Biochemical mechanisms in the respiration of the *Avena* coleoptile. *Arch. Biochem.*, 17 : 311-326.

Cook, R. P., and Alcock, R. S., 1931.—The effect of hydrogen ion concentration on some oxidations by *B. coli communis*. *Biochem. J.*, 25 : 523-533.

Damodaran, M., and Ramaswamy, R., 1940.—Succinic dehydrogenase in germinating seeds. *Current Sci.*, 9 : 319-320.

————, and Venkatesen, T. R., 1941.—Amide synthesis in plants. 1. The succinoxidase system in plants. *Proc. Indian Acad. Sci. B.*, 13 : 345-359.

Goddard, D. R., 1944.—Cytochrome c and cytochrome oxidase from wheat germ. *Am. J. Botany*, 31 : 270-276.

————, and Holden, C., 1950.—Cytochrome oxidase in the potato tuber. *Arch. Biochem.*, 27 : 41-47.

GREEN, D. E., LOOMIS, W. F., and AUERBACH, V. H., 1948.—Studies on the cyclophorase system. 1. The complete oxidation of pyruvic acid to carbon dioxide and water. *J. Biol. Chem.*, 172 : 389-403.

KEILIN, D., and HARTREE, E. F., 1937.—Preparation of pure cytochrome c from heart muscle and some of its properties. *Proc. Roy. Soc. (London) B.*, 122 : 298-308.

KREBS, H. A., and EGGLESTON, L. V., 1940.—The oxidation of pyruvate in pigeon breast muscle. *Biochem. J.*, 34 : 442-459.

————, SMYTH, D. H., and EVANS, E. A., 1940.—Determination of fumarate and malate in animal tissues. *Biochem. J.*, 34 : 1041-1045.

LARDY, H. A., 1949.—Respiratory enzymes. Revised edition. Burgess Publishing Company, Minneapolis, Minn., U.S.A.

LATIES, G. G., 1949.—The oxidative formation of succinate in higher plants. *Arch. Biochem.* 22 : 8-15.

LEVY, H., and SCHADE, A. L., 1948.—Studies in the respiration of the white potato. 2. Terminal oxidase system of potato tuber respiration. *Arch. Biochem.*, 19 : 273-286.

LUGG, J. W. H., and OVERELL, B. T., 1948.—"One" and "two-dimensional" partition chromatographic separations of organic acids on an inert sheet support. *Aust. J. Sc. Res. A.*, 1 : 98-111.

OHLSSON, E., 1921.—Die Abhängigkeit der Wirkung der Succinodehydrogenase von der Wasserstoffionenkonzentration. *Skand. Arch. Physiol.*, 41 : 77-100.

OKUNUKI, K., 1939.—Über den Gaswechsel der Pollen. 3. Weiters Untersuchungen über die Dehydrasen aus den Pollenkörnern. *Acta Phytochim.*, 11 : 65-80.

POTTER, V. R., 1941.—Studies on the mechanism of hydrogen transport in animal tissues. 4. The succinoxidase system. *J. Biol. Chem.*, 141 : 775-787.

————, and DUBOIS, K. P., 1943.—Studies on the mechanism of hydrogen transport in animal tissues. 6. Inhibitor studies with succinic dehydrogenase. *J. Gen. Physiol.*, 26 : 391-404.

ROBBIE, W. A., 1946.—The quantitative control of cyanide in manometric experimentation. *J. Cellular Comp. Physiol.*, 27 : 181-209.

————, and LEINFELDER, P. J., 1945.—Calcium cyanide solutions as constant sources of hydrogen cyanide gas for animal experiments. *J. Ind. Hyg. Toxicol.*, 27 : 269.

WIELAND, H., and FRAGE, K., 1929.—Über den Mechanismus der Oxydations vorgange. 20. Beiträge zur Kenntniss der Bernsteinsaure-dehydrase. *Ann. Chem.*, 477 : 1-32.